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# Fast access to positional $^{13}\text{C}$ contents of cholesterol in low amounts using an adiabatic 2D NMR experiment: Application to cheese authentication

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## Résumé

Cholesterol plays a central role in many biochemical processes and is linked to several diseases. In previous studies done by our team, an NMR method for  $^{13}\text{C}$  position-specific isotope analysis of cholesterol was developed using an optimized refocused adiabatic INEPT (Insensitive Nuclei Enhanced by Polarization Transfer), with high precision of the order of few per mil, as requested for isotopic analyses 1, 2. However, this method required 80 mg of cholesterol in order to be carried out in a reasonable time, while in most biological matrices, it is not practical to obtain such a quantity. Therefore, the objective of our work was to develop a new approach for the high-precision (few permil) isotopic analysis of small quantities of cholesterol. In order to increase the SNR per unit of time we chose to use symmetric and adiabatic Heteronuclear Single Quantum Coherence (HSQC) 2D NMR sequence, and to reach the target precision, adiabatic pulses were incremented. Moreover, several factors such as the use of Variable Recycling Times (VRT), Non-Uniform Sampling (NUS), and Linear Prediction (LP) were evaluated and optimized in order to reduce the NMR experiment duration. Additionally, number of increments and spectral range were also adjusted<sup>3</sup>. We demonstrated that the developed method can be used even with 5 mg of cholesterol while obtaining a repeatability lower than 2 permil on the 24 non-quaternary carbon atoms of the molecule in 31 min instead of 16.2 hours with our previous INEPT method. This allowed to expand the  $^{13}\text{C}$  position-specific isotope analysis to matrices low in cholesterol, such as dairy products. Then, cheese samples were collected and cholesterol was quantitatively extracted and analyzed by the newly developed sequence. Molar fractions of cholesterol  $^{13}\text{C}$  isotopomers obtained from HSQC spectra were used as inputs in the construction of classification models for cheese authentication. Cheese samples were successfully classified according to their producing species, geographical origin and type.

## References

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\*Intervenant

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